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Circuit Modeling of the Electrical Impedance: Part II Normal Subjects and System Reproducibility

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Abstract

Part I of this series showed that the 5-element circuit model accurately mimics impedances measured using Multi-Frequency Electrical Impedance Myography (MFEIM), focusing on changes brought on by disease. The present paper addresses two requirements which must be met if the method is to qualify for clinical use. First, the extracted parameters must be reproducible over long time periods such as are involved in the treatment of muscular disease, and second, differences amongst normal subjects should be attributable to known differences in the properties of healthy muscle. It applies the method to five muscle groups in 62 healthy subjects, closely following the procedure used earlier for the diseased subjects. Test-retest comparisons show that parameters are reproducible at levels from 6 to 16% (depending on the parameter) over time spans of up to 267 days, levels far below the changes occurring in serious disease. Also, variations with age, gender and muscle location are found to be consistent with established expectations for healthy muscle tissue. We conclude that the combination of MFEIM measurements and 5-element circuit analysis genuinely reflects properties of muscle and is reliable enough to recommend its use in following neuromuscular disease.

Keywords

muscle; electrical impedance; circuit modeling; neuromuscular disease

1. Introduction

In the accompanying paper we showed that the parameters of the 5-element circuit model are sensitive indicators of the effects of neuromuscular disease, by following parameter values as disease progressed and by examining the ratios of parameters to those for healthy subjects (Shiffman and Rutkove 2012, referred to as “Part I” in what follows.) That paper briefly described the measuring system and analytic procedure for extracting the parameters from the impedance data and provided references to technical details on the apparatus *per se*. The present paper deals with two related issues: first, how do we know the overall system is reliable over the very long time spans involved in dealing with neuromuscular disease, and second, what evidence is there that the parameters so extracted really represent muscle. The approach to the first question is to use traditional test-retest analysis on subjects whose muscles can be shown to be stable over appropriate time spans. The tests must allow for inadvertent variations in the properties of entire measuring system, and in particular effects at the electrode-skin interfaces. Section 3.2 is devoted to this topic, making use of data from a “control group” of healthy volunteers, obtained during the same period as for the patients with neuromuscular disease.

With regard to the second question, the results for the that group are also used to demonstrate that the parameters are governed by the properties of the tissue deep within the

musculature, indeed the full cross-section of the limb, despite reliance on surface electrodes alone, and it is shown that variations of the parameters within the group are in accord with established properties of healthy muscle, in particular their age dependences. Finally, Part I made heavy use of ratios between parameters for subjects with disease and healthy subjects of the same age, and the latter are provided by the measurements reported here for the control group.

2. Locale and Subjects

All measurements were done at the Department of Neurology of the Beth Israel Deaconess Medical Center in Boston and were conducted in accordance with Institutional Review Board regulations of the Center. Sixty-two volunteer subjects showing no evidence of disease were studied (31 men ages 19-91 and 31 women ages 21-90), all having signed the Center's Informed Consent forms.

3. Summary of the method

3.1 The impedance measurements and overall analysis scheme

For completeness and ease of reference, fig. 1 shows the 5-element circuit and contrasting examples of the reactance and resistance data for the MG's of a healthy subject and of a patient with highly developed ALS. The solid lines demonstrate the quality of the least-squares fits to the circuit model and its ability to represent the wide range of muscle conditions which were encountered in the survey of Part I, from health to severe disease. (The text will make occasional reference to the more commonly adopted 3-element circuit, namely the circuit shown in the inset with elements c_2 and r_3 removed.)

A detailed description of the experimental method and the impedance measuring apparatus is given by Shiffman et al. (2008), and a point meriting particular emphasis in the present context is the positioning of the current injecting electrodes far from the voltage electrode array. This insures that the current flow lines in the voltage measuring region are determined by the anatomy of the subject, uninfluenced by the unavoidable spreading in the neighborhood of the injecting electrodes. Consequently there is no need for those to be repositioned precisely in making test-retest comparisons (Rutkove et al., 2005a, 2006; see also Grimnes and Martinsen, 2007). But estimates of the reproducibility of the system will certainly be affected by failure to carefully reposition the *voltage* electrodes, and it must be stated that in acquiring the impedance data on which this retrospective analysis is based no instructions were given beyond verbal specification of distance from accessible landmarks. In a controlled study that would be corrected by the use of tattoos. Similarly, variations in subject posture can also contribute to irreproducibility, in particular as they change joint angles, and here also only rudimentary precautions were taken. In these and more fundamental respects to be discussed below the estimates of reliability of the measuring system undoubtedly *understate* what would be found in a statistically well-planned program.

Some subjects were examined on more than one date, the number of muscles examined per visit ranging from 1 to 8 (depending on the time available for the subject), for a total of 88 visits and 437 separate sets of impedance measurements. And as in Part I, a "set" consisted of 4 to 7 placements of the proximal voltage electrode (7 for the quads, 5 for the biceps, TA and MG, and 4 for the forearm), and the frequency range was from 3 to 300 kHz. A uniform procedure was followed for analyzing the R and X data: (i) the R(f) and X(f) data for each selected V-electrode combination were least-squares fitted to the theory for the circuit model, giving the 5-parameters for the associated segment of the muscle. For brevity we designate these by $p_i(z)$, z being the length of the segment (cf. fig. (3) of Part I); (ii) for each p_i its dependence on z was plotted and examined for smoothness and proper extrapolation to

zero length, and (iii) each such $p_i(z)$ set was fitted to a quadratic in z , and the fitted value at maximum z was adopted as the value of the parameter for the muscle in question. An example of this procedure is given in fig. (2). In what follows the paper discusses reproducibility of the overall easurement/analysis system, bilateral symmetries, age dependences, and upper vs lower extremity values, for which the required parameter values were obtained by culling members from the full 437 parameter-set collection as appropriate.

3.2 Reliability of the parameter extracting system

The ultimate object of this research is to apply MFEIM and the 5-element circuit analysis to the assessment of disease, and to that end one will need realistic estimates of the error bars which should be placed on the derived parameter values. Those estimates should reflect all possible sources of uncertainty, from inconsistencies in properties of the electrode-skin interfaces to ambiguities in the least-squares fitting procedure, and this requires that test-retest measurements on healthy human subjects be conducted under conditions essentially identical to what applies to measurements on diseased subjects. In particular, they should involve time spans of many months, and that presents a serious problem. To assess reproducibility of the *system* one must include data only for muscles which one is confident have not changed over such long time spans, but there are virtually no objective measures of muscle status which could be used to verify that condition at the levels of discrimination which will be required. To be sure, strength tests are often used in muscle physiology studies, but these usually require pre-test training of the subjects, clearly inapplicable in a retrospective analysis such as this. Similarly, MRI and other sophisticated imaging techniques were not applied, and given their expense, probably could not have been.

We have turned therefore to the emerging evidence that a highly sensitive noninvasive and objective indicator of muscle stability is likely to be the impedance itself, and when coupled with the right choice of frequency this is not as logically circular as it might at first appear. As discussed elsewhere (Shiffman et al., 2008), the major sources of error in MFEIM lie in voltage-electrode/skin-interface effects, which are serious mainly at very low frequencies, and stray capacitance and leads-disposition (mutual inductance) effects, which are serious at very high frequencies. But neither is a major problem at mid-range frequencies such as 50 kHz. Secondly, the 50 kHz phase (" θ_{50} ") is known to be sensitive to muscle condition, as illustrated by a host of investigations. A conservative approach therefore is to assume that if in a given case θ_{50} has changed then the involved muscle tissue has also changed. Therefore in collecting test-retest ratios for the purpose of assessing the reproducibility of the system the results for that case should be excluded.

Such a rigid criterion is impractical, however, and the condition has been relaxed to allow inclusion of test-retest parameter ratios if the 50 kHz phase ratio falls between 0.9 and 1.1 . This is far from a perfect criterion, but its failings have the effect of interpreting real muscle changes as *system* inconsistencies, i.e. the criterion leads to *understatements* of the reproducibility of the system. (A specific example is given in section 4.1, below.)

3.3 Girth normalization

In making comparisons between parameters for different subjects or for different muscles of the same subject it is critically important that differences in muscle dimensions be taken into account. Part I describes the "girth normalization" process which must be applied to the parameters as the primary step in doing so, and unless otherwise indicated that procedure has been applied throughout in what follows. For example fig. 2 shows the more familiar "raw" parameter values, as do the parameters in the caption to fig. 1.

4. Results

4.1 Overall System Reproducibility and Error Bar Estimates

Figure 2 shows the dependence of the parameters on the length “z” of the muscle segment involved, for two sets of measurements on the left quadriceps of a 65 year old healthy woman, taken 169 days apart. Subplots (a)-(e) show the before and after z-dependences for each of the parameters, derived from 14 independent sets of impedance data and their least-squares fits to the circuit model. The agreement of the curves for the two dates is very good, and all extrapolate to values close to zero at $z = 0$, arguing that they indeed represent properties of the subject *per se*. To quantify such differences as do occur, the ratios of the z-averages for the two visits are given in each plot. The sixth subplot of the figure shows the dependences of the 50 kHz phase on the length of muscle segment, and the close agreement between the two data sets is taken as *prima facie* evidence that no significant changes in muscle condition occurred; the mean 50 kHz phase ratio was well within the 0.9 to 1.1 range, so the data for this subject certainly qualify for inclusion in the pool used to assess the system reproducibility.

An illustration of the conservative nature of the inclusion criterion is the impedance of the TA of a 21 y.o. healthy male (not shown), measured over an almost equally long interval, 161 days. On the whole the picture of the z-dependences is virtually the same as in fig.2, and in fact the z-averaged parameter ratios for all but $1/c_2$ were actually better -- 0.97, 1.0, .99, and 1.0 -- but the ratio for $1/c_2$ was 1.19, decidedly worse. The 50 kHz phase dropped by 6%, and (pending assignment of error bars) one could conclude that there was a real change in the muscle tissue, apparently due to a change in the single parameter $1/c_2$. Nevertheless, according to the $0.9 \leq \theta_{50} \leq 1.1$ criterion the parameter ratios were all allowed into the system reproducibility pool, despite evidence of actual change in muscle condition.

Table 1 summarizes the results for 55 similar test-retest ratios for intervals up to 267 days (mean ≈ 100 days), showing the mean values and standard deviations for each of the parameters of each muscle group. (14 others failed to satisfy the θ_{50} criterion.) The overall average for all qualifying ratios is 1.04 ± 0.03 (SD), treating all qualifying subjects and all five muscles equally, and their distribution is approximately Gaussian, centered slightly above ratio 1.0. (Except for $1/c_2$ the individual parameters all satisfy the Bera-Jarque test for normality, with $\alpha = 0.05$.) There evidently has been a slight upward shift with time ($p \approx 2 \times 10^{-4}$, $\alpha = .05$, 2 sided) for which there would be no explanation except possibly a drift in the value of the reference resistor of the lock-in amplifier system (cf. fig 3 of Part I.)

In any case, what is sought here are estimates of overall system reproducibility as they apply to individual parameter determinations, while the table gives these for the ratios. Since one can safely assume that the system errors are the same for test and retest data, the standard deviation for the ratios should be 2 times the common standard deviations for the numerators and denominators. For the purpose of estimating anticipated uncertainties for an individual measurement one should therefore divide the SD for the selected muscle and parameter in the table by that factor. But as a compact summary statement we can instead take the weighted average SD over the 5 muscles and then apply the correction. The results are then 6, 6, 16, 11 and 12% for r_1 , r_2 , r_3 , $1/c_1$ and $1/c_2$ respectively.

4.2 Bilateral Muscular Symmetry

For most subjects the body is close to bilaterally symmetric, and one expects to find similar behavior for the impedances of the skeletal muscles. The only prior evidence for that is the work of Rutkove et al.(2005b) which shows that for a number of skeletal muscles the left and right 50 kHz phases can differ by about 10% (SD). Here we examine whether roughly the same holds for the 5-element circuit parameters, using (girth normalized) left-to-right

parameter ratios. The results for 63 same-day bilateral measurements are summarized in table 2 (which follows the format of table 1.) All parameter averages are t-test compatible with the null hypothesis that the mean value is 1.0, except the for r_3 , which is significantly different on the MG ($p = 0.003$, $\alpha = 0.05$, 2-sided) and marginally so on the quads ($p = 0.04$) The overall average, combining all subjects and all tested muscles, is 1.04 ± 0.19 .

4.3 Age dependences

It has been shown previously that the 50 kHz phase drops substantially with age (Aaron et al. 2006), and one expects to find corresponding trends in the parameters. The bar plot of figure 3 shows the results for the quads as representatives of what is found for the five muscle groups. The numbers in parentheses at the top of the r_1 plot are the bin populations, and the lines protruding from the tops of the bars indicate the SEM values. As seen, the population of the oldest group is only 5, so there the SEM calculation is a purely formal exercise. Putting the 80-100 year values aside then, the dependence of the parameters on age is roughly linear for all 5 muscles. Table 3 summarizes that situation via the slopes of least-squares-fitted lines (expressed in percent per year) along with the r-squared values for each fit. It is important to emphasize that the trends shown here should be distinguished from the well known loss of muscle mass with advancing age, since that has (presumably) been taken into account via the use of girth normalization procedure.

4.4 Similarities and differences amongst skeletal muscles

Once age is taken into account there is considerable agreement between (girth normalized) parameters for different muscles on the same limb, but substantial differences between those on the upper and lower extremities. Here the designation “lower extremities” refers to the three leg muscle areas we examined, i.e. the medial gastrocnemius, tibialis anterior and quadriceps, and the collective mean values and standard deviation are calculated from their means and SEM's. For example, mean r_1 values for males in the 60-80 age group are 213 for the quads, 230 for the TA's and 220 for the MG's, whose average is 220 with a (fractional) SEM of 2.5 %.

Similar calculations have been made for the other parameters and for the “upper extremities,” meaning the forearm finger flexors and the biceps, and for both genders. Since some of the parameters are much smaller than others, it is essential to examine the upper- to-lower ratios rather than differences, and the results are shown in figure 4. There the points are averages over both genders, and the different age groups are represented by different symbols, displaced slightly in the left-right direction for clarity.

The most striking result is that (apart from r_1) the ratios are larger than one, i.e. the (girth normalized) parameters for the upper extremity muscles are substantially larger than those for the lower extremity muscles. There are only small differences between the age groups, and averaging over the “cellular parameters” r_2 , r_3 , $1/c_1$ and $1/c_2$ one finds an overall “arms to leg” ratio of 1.66 ± 0.16 (SEM), certainly significantly different from 1 ($p \approx 0.005$, two sided.) Also, differences between males and females are marginal, i.e. 1.51 ± 0.14 for the males and 1.81 ± 0.13 for the females. But equally striking is the contrast between the r_1 ratios and the others, which corroborates the difference in character already evidenced by its almost total insensitivity to disease (cf. Part I.)

It goes without saying that in calculating these ratios it is imperative that girth normalized parameters be used, since the upper and lower extremity muscles are so different in their cross-sectional areas. By the same token, a systematic error in the girth normalization procedure would affect larger girth muscles more than smaller girth ones (or perhaps *vice versa*) *uniformly* across the full set of parameters, r_1 to $1/c_2$. In that respect the exceptional

behavior of the r_1 ratio has a subsidiary importance, a point to which we will return in section 5.5

4.5 Gender effects

Gender differences are similarly best examined by considering female to male parameter ratios for each muscle, and Table 4 shows the results for the combined age groups.

5. Discussion

5.1 Reproducibility

A principal result of this paper is the set of reproducibility figures for the parameters of the circuit model, i.e. 6, 6, 16, 11 and 12% for r_1 , r_2 , r_3 , $1/c_1$ and $1/c_2$ respectively. These represent estimates of inconsistencies inherent in a single application of the combined impedance measurement and circuit analysis scheme, independent of muscle condition, and they should be multiplied by 2 to estimate the errors applicable to the difference between two measurements. To put the results of so doing in appropriate context, we note that according to Part I the impact of serious disease can be even orders of magnitude larger. Examples include a patient with Charcot-Marie-Tooth disease (CMT), where the $1/c_1$ diseased-to-normal ratio for the quads was 11. This corresponds to a diseased-to-normal difference almost 65 times larger than the system uncertainty for that parameter, and for r_2 the figure was over 170. These are large but not exceptional results; corresponding figures for the TA of an axial neuropathy patient were 100 for r_1 and over 40 for $1/c_1$ and $1/c_2$, and in the case of an ALS patient, the diseased-to-normal differences for the capacitance parameters for the quads were just under 50 times larger than the experimental uncertainty.

The overall assessment is that in diseases serious enough to warrant medical attention the parameter changes are so large as to be more reasonably described in terms of ratios rather than differences. But however expressed, the presently achieved levels of reproducibility are certainly encouraging for use of the technique in the clinic. In that context it is important to point out that the system uncertainties just given are based on figures which very likely *overstate* the irreproducibility of the system (cf. section 3.2). In particular they combine the effects of random electrical noise and “hidden” changes in tissue properties occurring during the lengthy test-retest period. They do not take into account the ability to reduce the random component by averaging over several determinations for each patient at each visit (or simply increasing the lock-in amplifier filter time constant.) But more importantly, those figures represent standard deviations of the distributions of individual test-retest ratios, while what may be more relevant is the usually much smaller standard deviation of the *mean* values of such distributions, as in a placebo-based drug test.

5.2 Resistivity at “zero” frequency

In several respects these “control group” results strongly support the view that the overall scheme reflects true properties of muscle tissue, even though only surface electrodes are employed. A prime example is offered by relative independence of r_1 with respect to age, which makes it appropriate for comparisons with muscle resistivities in the literature. We note first that the girth-normalized values of the resistance parameters have dimensions of resistivity (ohm-cm) and could justifiably be referred to as “effective resistivities”. Secondly, r_1 is the model's extrapolation of the resistivity to true zero frequency, whereas much of what is found in the literature is obtained from logarithmic plots and *ad hoc* extrapolations, or simply an average over what is regarded as an adequately low frequency range. Nevertheless comparison with the survey by Faes et al. (1999) is informative. The range reported there is 170 to 240 ohm-cm, whereas the present work gives 199 ± 19 ohm-cm (as averaged over the

five muscles for both genders and all ages.) The agreement is very good, given the differences in technique and frequency range used by the various investigators.

5.3 bilateral symmetries

With respect to possible left-right asymmetries, we note that the healthy subject group is likely to have many more right-handed than left-handed members, just as in the general population, and in fact 18 out of the 25 mean values in Table 2 are larger than 1.0. However there is no reasonable evidence for statistically significant left-right differences in any particular parameter, with the single exception of the r_3 ratio for the medial gastrocnemius ($p = 0.002$, $\alpha = .05$, 2-sided.) While a variety of left-right differences have been reported in the muscle physiology literature, these involve either mass/strength or neural excitation issues, neither of which should be reflected in the girth-normalized, relaxed muscle parameters presented here.

5.4 Age dependences of the parameters

That the parameters are substantially dependent on age might come as no surprise, since loss of muscle mass is common with increasing age and there is a complex of more subtle changes as well. However mass loss should play only a small role in the *girth-normalized* parameters of fig. (3) and table 3, certainly not enough to explain the factors of 2 or more between 90 year and 30 year old subjects. To be sure, in keeping with the remarks of section 2.4 of Part I one must consider that the normalization scheme may be flawed when applied to the oldest subjects, by failing to include intrusions of fat and connective tissue. Indeed if there were appreciable intrusions, the scheme would overstate the cross sectional area of the *muscle* tissue and hence exaggerate the parameters, as might well appear to be the case for the over eighty group. However this argument is countered by the fact that such an error in the girth normalization scheme would affect all the parameters uniformly. Yet fig. 3 shows very different age dependences for r_1 and r_3 , as compared to r_2 , $1/c_1$, and $1/c_2$, and Table 3 shows that there are considerable differences in rates of change amongst these last three as well. All of these strengthen the hypothesis that the age dependences found for the girth normalized parameters reflect changes in fundamental physiological properties of the tissue beyond simply changes in muscle mass.

5.5 Evidence for different muscle types

Figure 4 offers evidence that there are major differences between upper and lower extremity parameters (except for r_1), and the use of ratios shows that this is equally true for the small parameter $1/c_1$ as for the large parameters r_2 , r_3 and $1/c_2$. Furthermore, the error bars show that the variations in parameter values within each group are on the whole much smaller than the differences in parameter values between the two groups. Three conclusions are suggested by these results. First a good case can be made that there are genuine differences in the character of the muscles of the two extremities, differences to which the cellular parameters are sensitive. The fact that the two groups of muscles can be described as “weight bearing” and “non-weight bearing” comes naturally to mind, but in any case the data are challenging and the impedance measurements should be repeated under more controlled conditions. Second, figure 4 offers strong support that the girth correction procedure is essentially correct, the point here being that in dealing with musculatures as massive as the quads and as slender as the forearms finger flexors, errors in compensating for the cross-sectional area will be much more evident than, say, in comparing girth-normalized values of r_1 to resistivity values in the literature.

5.6 Gender differences

Biopsy and MRI studies show that fibres from men have larger cross-sectional areas than those from women of the same fitness class (Miller et al. 1992). If, as often stated, the number and constitution of the fibres is the same, then all the female impedance parameters should be correspondingly larger. Table 4 offers evidence for this in the age-averaged female to male ratios, in which 21 of the 25 entries are equal to or larger than one. Also, the histogram (not shown) for the entire collection is definitely skewed to high values of the ratio and the t-test gives $p = 6 \times 10^{-4}$, one sided. No clear statistical picture emerges for individual parameters, but taken together the two capacitance parameters give $p = 0.009$ (single-sided). Certainly the largest gender differences are found for $1/c_1$, where the female parameters are on average almost 40% larger than those for males. Since c_1 is presumably proportional to the fiber surface area, shrinkage should raise $1/c_1$, as is found. Whether such a large effect is to be expected and why there is so much variation amongst the other ratios remains to be studied.

5.7 Interpretation of the model

The 5-element model has been shown to be a compact and reliable representation of the impedance of skeletal muscle over a wide range of frequencies, whose parameters evolve smoothly and systematically as health changes from normal to severely diseased. However that should not be taken to mean that individual parameters of the circuit can unambiguously be identified with specific anatomical structure in the musculature. For one thing, it is well known that different circuit topologies can have exactly the same impedance functions, in which each of the parameters of one circuit is a well defined combination of those from the other. And above all it is extremely unlikely that any mathematical expression with such a small number of adjustable parameters could possibly represent anything as anatomically complex as muscle tissue. The most one can expect is that some functional or physiological characteristics will more strongly affect one parameter than others -- or perhaps more weakly.

The outstanding example of the latter is the insensitivity of r_1 to disease (cf. Part I), which we now find is matched by a very weak dependence on normal aging. One should add to this the findings of Kashuri et al. (2007), that r_2 , r_3 , c_1 , and c_2 are monotone functions of the force-time integral during voluntary isometric contractions (of the forearm muscles), while r_1 is not. Instead it rises very quickly at the onset of the contraction and remains constant until the muscle relaxes, just as expected for retrograde vascular flow driven by the laterally expanding fibres (Tschakovsky and Sheriff, 2004). Since r_1 is the zero-frequency impedance of the circuit, it can only represent current paths which do not encounter any cell membranes, but that does not mean that it represents the *entire* extracellular fluid, as is often assumed. For example the Kashuri results support the suggestion made in Part I that r_1 largely represents only the vascular part. The otherwise puzzling difference between the upper to lower extremity ratio for r_1 and for the other 4 parameters (cf. fig 4) would then be construed as simply reflecting that r_1 has little to do with muscle. (It might appear that in this scenario the much larger interstitial component of the extracellular fluid is unaccounted for, but it is largely concentrated in the endomysia of the fibres and would flow “through” the fibre membrane, appearing as a factor determining r_2 .)

The purpose here is not to claim that this re-identification of the circuit elements is anatomically justified, but to point out that it would have absolutely no effect on the theoretical impedance and hence on the quality of its fit to the data. But if verified it could have major effects on the interpretation of the impedance measurements, particularly in dealing with diseased subjects. That would apply even more to anatomically identifying the

“deep” parameters r_3 and $1/c_2$, and more focused and controlled studies extending over a wider range of frequencies will no doubt be needed to do so.

6. Summary

The major conclusions of this study are that parameters of the 5-element circuit model genuinely reflect properties of muscle, and that the combined measurement and analysis scheme is reliable enough for clinical use. The present retrospective survey establishes the orders of magnitude of the effects to be examined and the precisions which are attainable with conventional instrumentation, but it also raises questions which could guide further investigations. For example why is r_3 relatively unaffected by aging, and conversely if the substantial trends in the other cellular parameters are really independent of muscle mass, as claimed, then what do they signify? But putting aside such fundamental issues, it is important to recognize that the combination of MFEIM measurements and the 5-element analysis offers a reliable -- if only phenomenological -- new tool for objectively characterizing muscle status.

In this last connection it is also important to remark that much of the experimental complexity implied by the multi-electrode data of figure 2 (and fig. 3 of part I) and its attendant switching system is quite unnecessary for successful employment of the technique. Their role in this work was to implement various checks on the quality of the data and the reliability of the overall scheme, and in fact all the parameter values used in the trends and comparisons presented here were based on data from only the outermost two voltage electrodes. A more standard 4-terminal arrangement should suffice for use in the clinic, with the one proviso that the current injecting electrodes are placed far from the voltage sensing ones.

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Figure 1.

Frequency dependencies of R and X for the MG's of a healthy subject (circles) and a patient with advanced ALS (triangles). The solid curves show the fits of the 5-element model using the parameters $r_1 = 49.7$, $r_2 = 113$, $r_3 = 208$ (ohms), $c_1 = 0.040$ and $c_2 = 0.0065$ (μF) for the healthy subject and $r_1 = 20.8$, $r_2 = 143$, $r_3 = 132$ (ohms), $c_1 = 0.028$ and $c_2 = 0.0042$ (μF) for ALS patient. (These are uncorrected for muscle girth, cf. section 3.3 .) The inset shows the 5-element circuit.

Figure 2. 169 day test-retest of the MFEIM system and 5-element model analysis. Dependence of the circuit parameters on muscle segment length, z , along the quadriceps of a healthy 65 year old woman. The units are ohms for the three resistors, inverse microfarads for the capacitors and degrees for the 50 kHz phase (The parameters are “raw” values, i.e. uncorrected for muscle girth; cf. section 3.3 .)

Figure 3. Average girth-normalized parameters for 20 year-wide age bins for the quads, plotted against the mid-bin age. The figures in parentheses near the top of the r_1 sub-plot are the bin populations. The protruding lines indicate the SEM values.

Figure 4.
Ratios of (group averaged) upper-to-lower extremity parameters for healthy subjects. circles: 20-40 age group; upright triangles: 40-60; inverted triangles: 60-80

Table 1Parameters ratios $\pm$ SD for test-retest intervals of up to 267 days

Muscle (n)	r_1	r_2	r_3	$1/c_1$	$1/c_2$	θ_{50}
Biceps (10)	1.03 $\pm$.07	1.0 $\pm$.09	1.09 $\pm$.28	0.94 $\pm$.17	1.0 $\pm$.12	1.0 $\pm$.05
Forearm (5)	1.05 $\pm$.07	1.08 $\pm$.07	1.35 $\pm$.33	1.14 $\pm$.31	1.08 $\pm$.26	1.0 $\pm$.07
MG (11)	1.03 $\pm$.10	1.04 $\pm$.09	1.10 $\pm$.23	1.00 $\pm$.15	1.08 $\pm$.17	0.99 $\pm$.06
TA (14)	0.99 $\pm$.09	1.00 $\pm$.09	1.03 $\pm$.15	0.98 $\pm$.14	1.04 $\pm$.18	0.99 $\pm$.04
Quads (15)	1.00 $\pm$.08	1.03 $\pm$.09	1.06 $\pm$.24	1.08 $\pm$.16	0.97 $\pm$.16	1.0 $\pm$.04
weighted avg.	1.02 $\pm$.04	1.02 $\pm$.04	1.09 $\pm$.11	1.02 $\pm$.08	1.02 $\pm$.08	1.0 $\pm$.02
overall weighted average, all qualifying muscles and subjects: 1.04 $\pm$.03						

Table 2Average left - right parameter ratios for measurements on the same day $\pm$ SD

Muscle	(n)	r_1	r_2	r_3	$1/c_1$	$1/c_2$	θ_{50}
biceps	(10)	1.05 ± 0.09	1.02 ± 0.11	1.07 ± 0.21	0.98 ± 0.17	1.0 ± 0.40	1.03 ± 0.10
forearm	(5)	1.03 ± 0.09	1.0 ± 0.13	1.06 ± 0.32	1.15 ± 0.14	1.29 ± 0.56	1.03 ± 0.10
MG	(11)	1.0 ± 0.11	0.99 ± 0.14	1.17 ± 0.15	1.13 ± 0.21	1.07 ± 0.21	1.0 ± 0.11
TA	(17)	1.02 ± 0.10	1.01 ± 0.06	1.07 ± 0.18	1.06 ± 0.15	1.07 ± 0.19	1.01 ± 0.08
quads	(20)	1.02 ± 0.09	1.05 ± 0.12	1.11 ± 0.23	0.95 ± 0.18	1.0 ± 0.2	0.98 ± 0.09
averages		1.02 ± 0.1	1.02 ± 0.11	1.1 ± 0.20	1.03 ± 0.18	1.05 ± 0.28	1.01 ± 0.09

Table 320–80 year age dependencies; parameter slopes (%/year) and r^2 values

	r_1	r_2	r_3	$1/c_1$	$1/c_2$
Quads	0.07 (.55)	1.4 (.99)	0.09 (.41)	1.3 (.99)	.70 (.91)
TAs	0.1 (.32)	1.1 (.99)	1.1 (.99)	1.5 (1.0)	1.9(1.0)
MGs	-0.3 (.8)	0.9 (.96)	-0.0001 (~0)	1.2 (.87)	1.4 (.99)
Biceps	0.4 (.76)	1.1 (.99)	-0.3 (.55)	1.6 (.97)	0.2 (.34)
Forearms	-0.1 (1.0)	1.0 (.95)	0.08 (.04)	1.1 (.98)	0.51 (.91)

Table 4

Female-to-male girth-normalized parameter ratios for all age groups

	r_1	r_2	r_3	$1/c_1$	$1/c_2$
MG's	0.92±4%	1.03±7%	1.18±12%	1.14±8%	0.93±13%
TA's	1.15±5%	1.34±5%	1.23±6%	1.51±8%	1.44±8%
Quads	0.89±3%	1.08±7%	0.95±7%	1.08±7%	1.17±7%
Biceps	1.27±5%	1.7±6%	1.01±6%	2.04±8%	1.19±7%
Forearms	1.0±3%	1.25±5%	0.95±5%	1.48±6%	1.02±7%